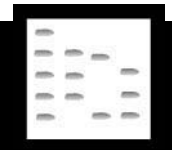
ALLERGENS IN PERFUMES: GAS CHROMATOGRAPHY–MASS SPECTROMETRY



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Perfumes (fragrance substances) are used in the formulation of consumer products to provide pleasure to the user and/or to mask malodours of some other ingredients in the products. Perfumes are also used in aromatherapy. A typical perfume may be composed of 10–300 substances selected from a battery of over 3000 synthetic and natural fragrance materials. It has been shown that approximately 2% of the general population is allergic to perfumes. Furthermore, perfumes have also been shown to be one of the major cause of allergic contact dermatitis from the use of cosmetics and toiletries. Besides cosmetics, the use of many other consumer products such as perfumed laundry detergents and dishwashers have also been implicated as the cause of perfume allergy in contact eczema patients.

Perfume allergy in contact eczema patients is diagnosed by patch-testing with a fragrance mix containing 1% each of geraniol, eugenol, isoeugenol, cinnamic alcohol, cinnamic aldehyde, α-amylcinnamic aldehyde, hydroxycitronellal and an extract from oakmoss – oakmoss absolute. However, only 50–80% of perfume allergy cases are diagnosed by this test. For the management of allergy, it is important to identify the fragrance allergen responsible for contact eczema in a patient, as this makes it possible for the patient to avoid the use of products containing the sensitizing allergen(s). To establish the identity of the fragrance substance responsible for perfume allergy in a contact eczema patient, it is recommended that the product(s) used by a patient should be analysed for the contents of fragrance allergens followed by patch-testing the patient with the relevant fragrance allergens present in the product.

Gas chromatography–mass spectrometry (GC-MS) is frequently used for the analysis of fragrance substances in essential oils. This approach is used for the identification and semiquantitative determination of the fragrance substances of interest in essential oils. In 1995, GC-MS was used for the identification and quantification of 10 selected fragrance substances including the seven chemically defined substances of fragrance mix in perfumes, eau de toilette, deodorants, creams, lotions, shampoos and other perfumed consumer products which may contain both natural as well as synthetic fragrance materials. The method was later modified slightly so that quantitative analysis of many more fragrance substances in perfumes or in perfumed products could be performed. This method, described in the present article, has been applied to the analysis of perfumes in various consumer products. To demonstrate the potential of the method for perfume analysis, example of analysis of fragrance substances in a deodorant and in an eau de toilette are presented here. Sample preparation methods for the GC analysis of fragrances in various types of consumer products is also described. The quantitative data on fragrance substances in various consumer products are reported in the publications described in the Further Reading section.

Target Fragrance Substances

The analytical method has been developed for the quantification of 21 fragrance substances which in relatively high concentrations are commonly used in the composition of perfumes, or which are established contact allergens:

1 geraniol: CAS registration number 106-24-1;
2 eugenol: 97-53-0;
3 isoeugenol: 97-54-1;
4 linalool: 78-70-6;
5 linalyl acetate: 115-95-7;
6 citronellol: 106-22-9;
7 cinnamic alcohol: 104-54-1;
8 cinnamic aldehyde: 104-55-2;
9 hydroxycitronellal: 107-75-5;
10 α-amylcinnamic aldehyde: 122-40-7;
11 α-hexylcinnamic aldehyde: 101-86-0;
12 α-isomethylionone: 127-51-5;
13 coumarin: 91-64-5;
14 piperonal: 120-50-7;
15 benzyl alcohol: 100-51-6;
16 benzyl acetate: 140-11-4;
17 benzyl benzoate: 121-51-4;
18 benzyl salicylate: 118-51-8;
19 Lilial®: 80-54-6;
20 Lyral®: 31906-04-4;
21 Hedione®: 24851-98-7.

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Approximately 1.0% (w/v) solutions of all of the substances in ethanol served as stock solutions. The stock solutions were stored in closed vials at 4°C and were used within 1 month.

Sample Preparation

Perfumes, Eau de Toilette, Aftershave and Deodorant Sprays

These products were approximately diluted in ethanol so that the concentrations of target fragrance substances were $\leq 0.1\%$. Depending on the concentrations of the target fragrance substances in a sample, it may be necessary to analyse several dilutions of the sample.

Shampoos, Creams, Lotions, Lipsticks, Face Powders and Deodorant Sticks

Perfumes from 1 g sample were extracted in 10 ml methanol at 60°C (to facilitate the extraction) followed by removal of matrix components by silica gel column chromatography. The extract was loaded on a 7×1.8 cm silica gel column, and the fragrance fraction was eluted with methanol. The perfume extract was stored at 4°C and analysed within 24 h.

Soap Bar and Laundry Detergents

Perfumes from 1 g sample dissolved in 50 ml water were extracted in 10 ml ethyl acetate employing liquid–liquid extraction. The perfume extract in ethyl acetate was centrifuged to remove any solid or aqueous contamination. The perfume extract was stored at 4°C and analysed within 24 h.

Dishwashing liquid

The method used for the extraction of perfumes from dishwashing liquids was the same as for shampoo.

GC-MS Analysis

MS Conditions

Electron impact ionization at 70 eV was used, scanning m/z 29–250 in 0.6 min.

Results

The method described here has been applied to the determination of 21 target fragrance substances in consumer products. The chromatographic separation of these 21 compounds employing GC is shown in **Figure 1**. Day-to-day variation of retention times of the fragrance substances is $< 0.5\%$. The detection limits of all of the target substances are ≤ 1 p.p.m., the calibration curves for all of the target substances are linear (coefficient of correlation > 0.995) in the tested concentration range 10–2000 p.p.m., the relative standard deviations of the determination of all of the substances are $< 11\%$. The recovery of all of the target substances from the spiked samples is 82–116%, and day-to-day variations of quantitative analysis for all of the substances are within 5%.

The reconstructed ion chromatogram obtained by GC-MS analysis of fragrance substances in a deodorant (undiluted) is shown in **Figure 2**. The fragrance substances in the product were identified by comparing the retention times of the GC peaks with those of the reference materials, as well as by comparing the spectra of the GC peaks with the reference spectra of standard compounds in the mass spectrum library. Followed by GC-MS identification, quantification of target fragrance substances in the sample is carried out with external standards.

Most consumer products contain many more fragrance substances other than the target compounds. The identification of these substances was only performed by comparing the mass spectrum of a GC peak with the mass spectra of reference compounds in the MS library. In this case, both the spectrum fit and spectrum purity of match of the unknown spectrum with those of library spectra were > 900. An example of identification of fragrance substances in an eau de toilette is shown in **Figure 3A–E**, where the results are divided in six windows for the clarity of peak identification. Confirmation of the identification of these substances and their quantification were performed where a reference material was available.

In some cases it is not possible to identify all the peaks because of the absence of mass spectra of the compounds in the mass spectral library.

Discussion

For the analysis of perfumes on a routine basis, GC-MS identification of the fragrance substances followed by quantification employing GC-flame ionization detection (FID) was found to be a more suitable approach. The main reason for this is that the use of GC-FID allows relatively rapid production of validated data. Thus, several relevant analysis recommended by quality assurance/quality control (QA/QC) protocol for a set of samples can be easily performed by GC-FID. Fulfilling the requirements of QA/QC protocol for the analysis by GC-MS is time-consuming, because it requires tuning and calibration of the MS at regular intervals and frequent cleaning of the ion source. The detection limits of the target

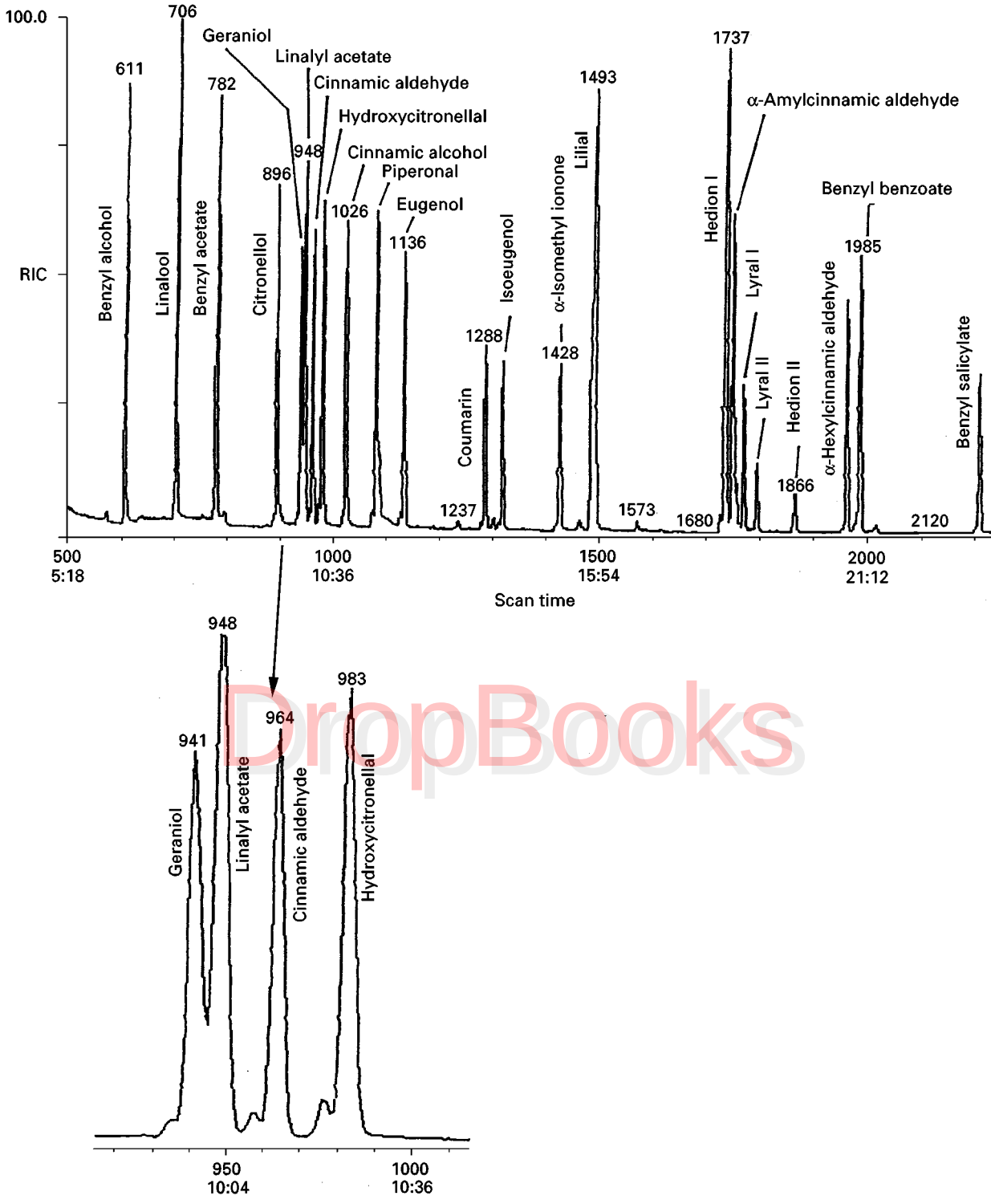

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Figure 1 GC-MS analysis of a mixture containing 83–117 p.p.m. of the 21 target fragrance substances. 50 m × 0.32 mm, 1.2 μm film thickness Chrompack fused silica capillary columns coated with CP-Sil-5CB, were used. 1 μL split injection; helium carrier gas flow 30 ml min^{-1}, column-head pressure 20 psi; injector temperature 300°C; column temperature program: 40–140°C in 4 min, thereafter 5°C min^{-1} to 280°C, 5 min at 280°C. 2 μL injection volume was used when the content of perfume in a sample was relatively low.

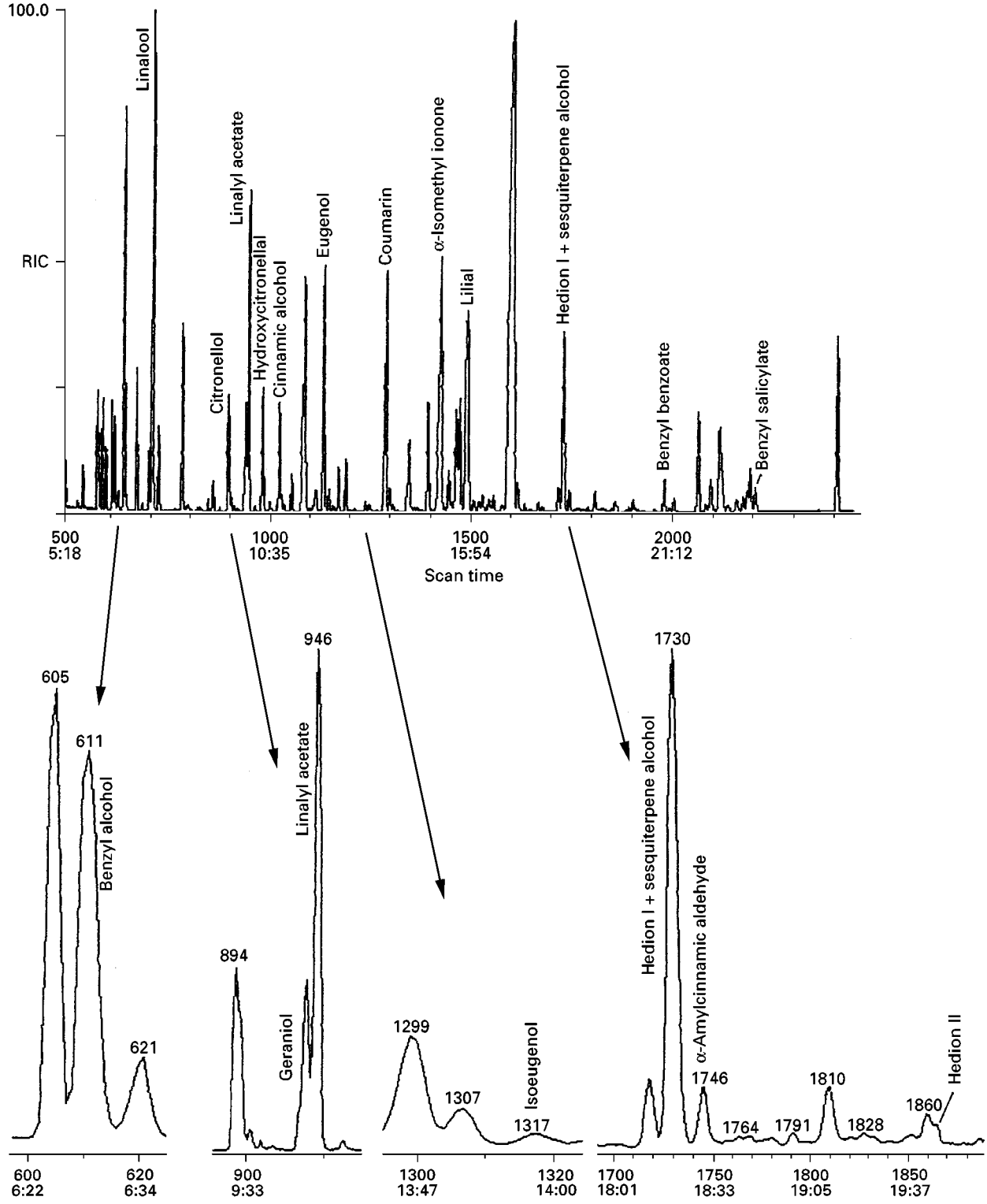

Figure 2 GC-MS analysis of the target fragrance in an undiluted deodorant. The following were present among the target fragrance substances: 102 p.p.m. benzyl alcohol, 1028 p.p.m. linalool, 141 p.p.m. citronellol, 136 p.p.m. geraniol, 614 p.p.m. linalyl acetate, 205 p.p.m. hydroxycitronellal, 183 p.p.m. cinnamic alcohol, 408 p.p.m. eugenol, 1051 p.p.m. coumarin, 7 p.p.m. isoeugenol, 319 p.p.m. α-isomethylionone, 291 p.p.m. Lilial®, 199 p.p.m. Hedion®, 68 p.p.m. α-amylcinnamic aldehyde, 101 p.p.m. benzyl benzoate and 112 p.p.m. benzyl salicylate. Quantification of Hedion® was performed by the analysis of 1 : 10 dilution of the sample, where no interference by the sesquiterpene alcohol present in the sample was observed.

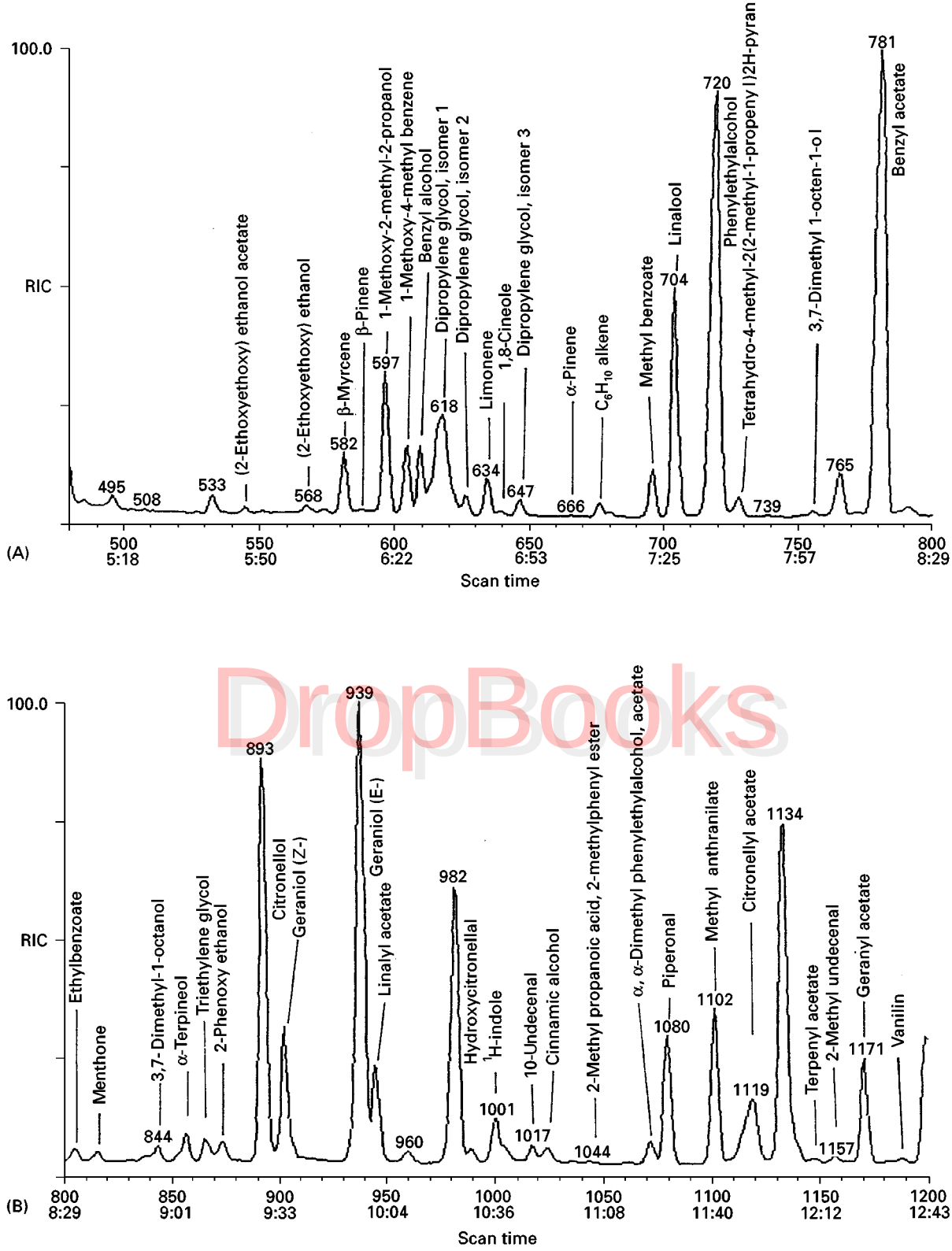

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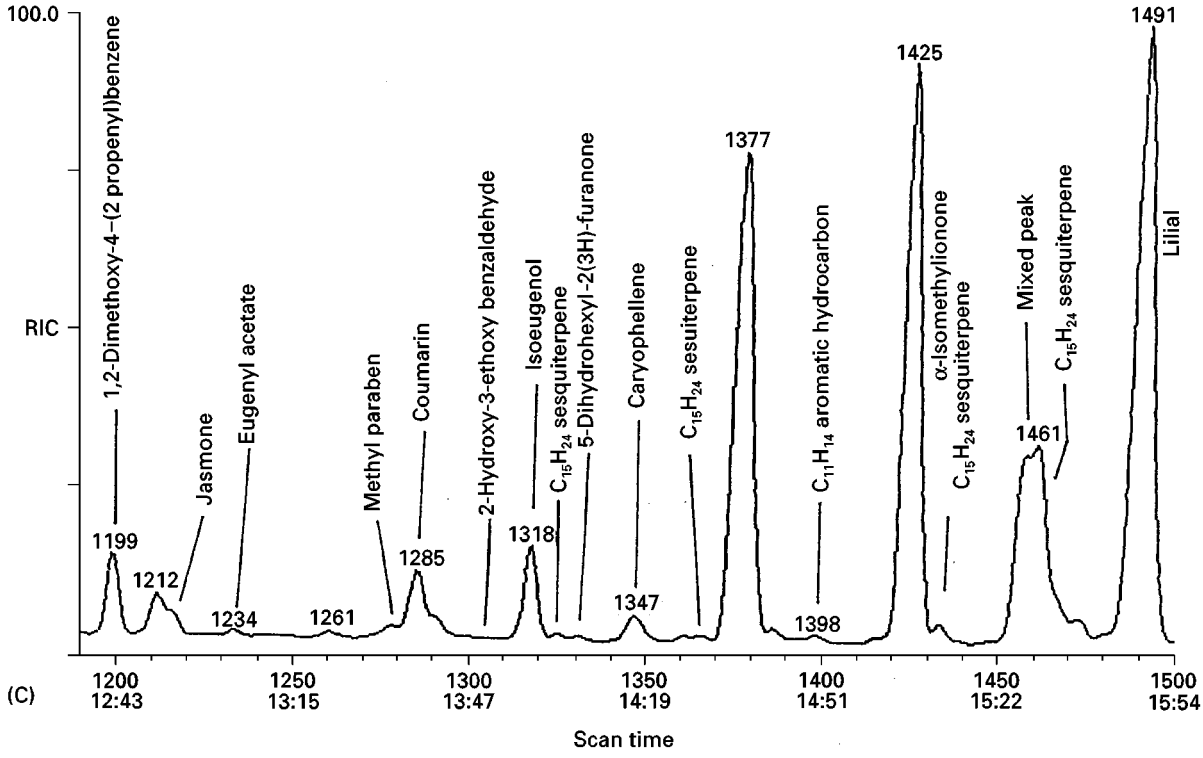
Figure 3 GC-MS analysis of an eau de toilette, diluted 1 : 10 in ethanol. The reconstituted ion chromatogram is divided in six windows (A–F) for the clarity of the compounds identified in the sample. Peaks with no name could not be identified.



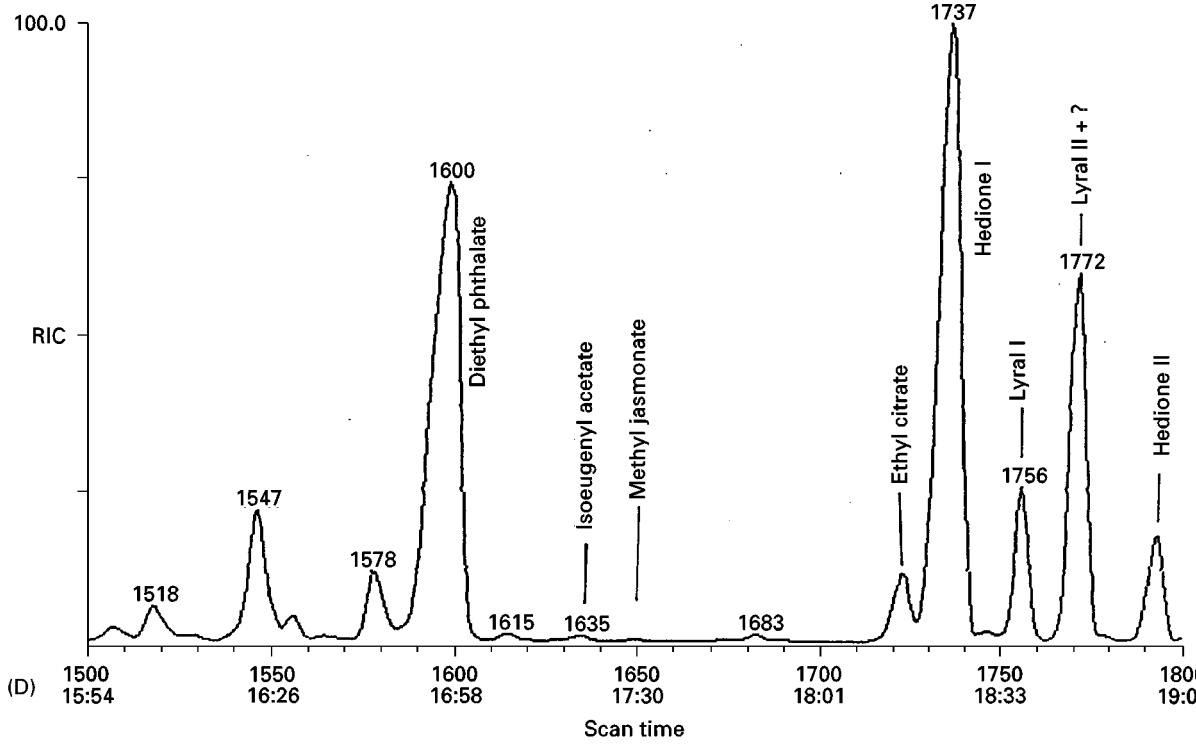
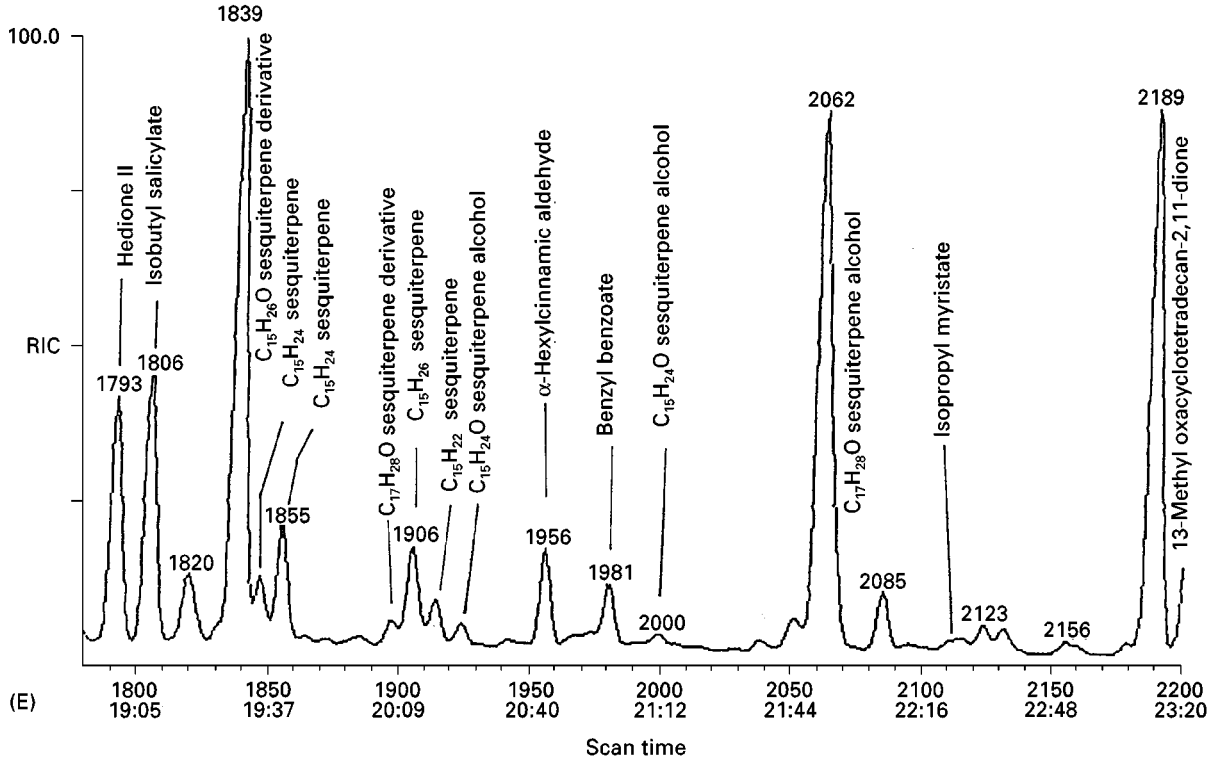


Figure 3 *Continued*



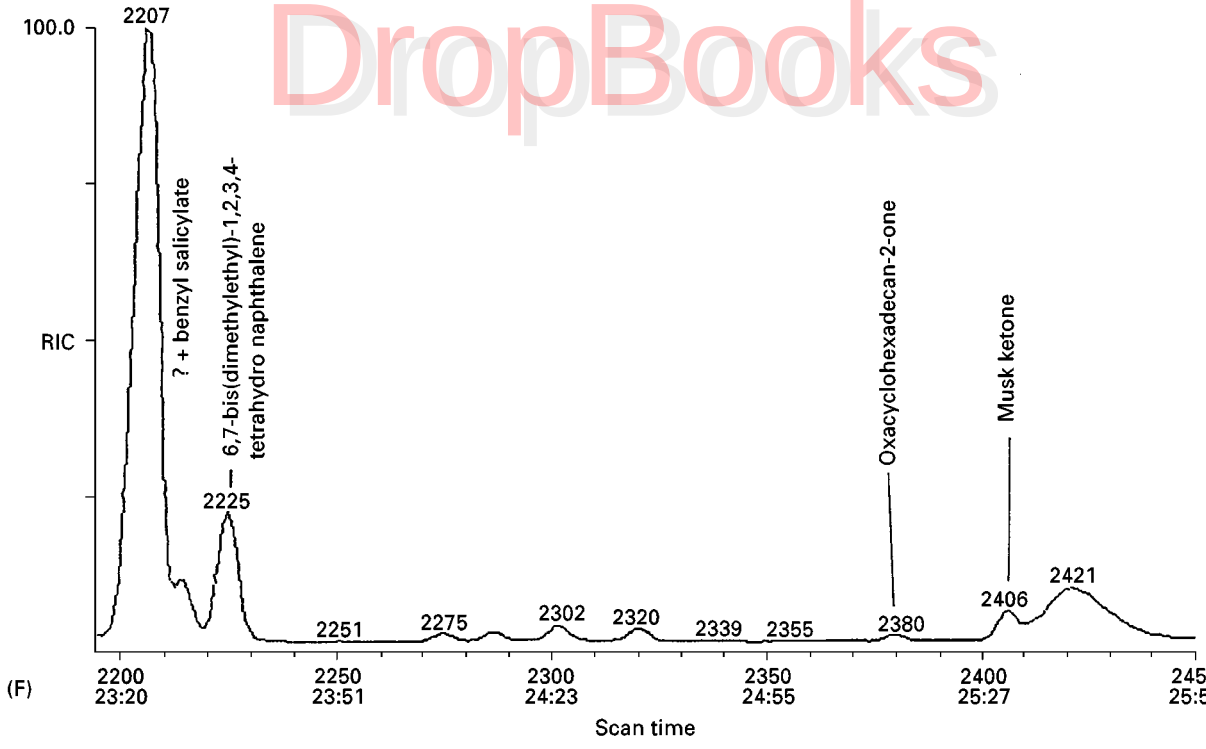


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Figure 3 *Continued*

substances by GC-FID, however, are 2–5 p.p.m. So, unless the quantification was required at 1 p.p.m. level, GC-FID was chosen for the determination of fragrance substances after prior identification by GC-MS.

Most of the fragrance substances in use, including the target fragrance substances, have a molecular weight < 250 Da. Therefore, the MS scan was performed only up to m/z 250. Occasionally, for example in the identification of musk ketone, it is necessary to scan masses up to 300.

Not all the fragrance ingredients in all tested products could be identified or quantified, in some cases due to interferences. Occasionally the GC peak of a relatively high amount of dipropylene glycol present in a sample overlapped the peak by benzyl alcohol; a C_{11}-alkyne interfered with the analysis of Lilial®; high amounts of triethyl citrate and/or a sesquiterpene alcohol ($C_{15}H_{26}O$) interfered with the analysis of Hedione® and relatively high amounts of Hedione® interfered with the analysis of α-amylcinnamic aldehyde. An unidentified compound was found to interfere with the analysis is benzyl salicylate. In most cases these problems could be solved by analysing diluted samples.

By using GC-MS, identification of 226 substances in deodorants has recently been reported. A structure–activity relationship (SAR) analysis of contact allergens revealed that 84 of the identified compounds possess at least one structural alert (chemical group) having sensitizing potential, and 70 belong to, or are susceptible to metabolize into, the chemical groups having sensitizing properties: aldehydes, ketones and α,β-unsaturated aldehydes, ketones or esters. The combination of GC-MS and SAR analysis could be helpful in the selection of substances for supplementary investigations regarding sensitizing properties.

Analysis of as many fragrance ingredients as possible in a perfumed product is of great importance for clinicians to establish the identity of contact allergens in each case. This information is also important for clinical research to investigate cross-reactions of fragrance allergens. The quantitative data on the fragrance ingredients in consumer products make a basis for exposure assessment that is a help for establishing threshold concentrations of fragrances for the elicitation of contact allergy.

Conclusions

Chemical analysis of perfumes and perfumed products is of great importance for the diagnosis and management of perfume allergy. The GC-MS/GC-FID method described here for the analysis of fragrance substances in consumer products has proved to be valuable to identify allergens in patients with contact eczema from the use of perfumes and perfumed products. Using GC-MS in combination with SAR it has been possible to identify several fragrance substances in perfumes which possess sensitizing potential.

See also: **II/Chromatography: Gas:** Column Technology; Derivatization; Detectors: General (Flame Ionization Detectors and Thermal Conductivity Detectors); Detectors: Mass Spectrometry; Detectors: Selective; Headspace Gas Chromatography; Theory of Gas Chromatography. **III/Flavours: Gas Chromatography: Sulphur Compounds: Gas Chromatography.**

Further Reading

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